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In vitro sensitivity of poultry *Brachyspira* isolates to essential oil components and *in vivo* reduction of *Brachyspira intermedia* in rearing pullets with cinnamaldehyde feed supplementation

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INTRODUCTION

Cecal enteritis due to *Brachyspira* infections tends to be chronic in laying hens. Limited availability of antimicrobial drugs for use in laying hens emphasizes the need for alternative control measures.

We tested *in vitro* the sensitivity of poultry *Brachyspira* isolates to essential oil components and in an *in vivo* experiment the effect of cinnamaldehyde feed supplementation on the colonisation rate of *Brachyspira intermedia* in rearing pullets.

MATERIALS AND METHODS

A broth microdilution method was used to determine the antimicrobial susceptibility of 20 *B. intermedia* field isolates from laying hen flocks to components of essential oils (EO).

RESULTS

Minimal inhibitory concentration (MIC) distributions, obtained for eight EO components, were all monomodal. Cinnamaldehyde had the lowest MIC values (40-80 mg/l), followed by nerolidol, capsaicin, carvacrol and thymol (80-320 mg/l), eugenol (160-640 mg/l) and linalool (320-1280 mg/l). The MIC ranges of piperine were mostly above the test-range of 1280 mg/l (Table 1).

Table 1

Distribution of minimal inhibitory concentrations (MICs) of eight essential oil components on 20 *Brachyspira intermedia* isolates from laying hens in Belgium and the Netherlands

	Number of isolates with MIC (mg/l) of						
Component	40	80	160	320	640	1280	>1280
Carvacrol		6	5°*	9			
Thymol			10°*	10			
Linalool				2°*	7	11	
Nerolidol		1	19°*				
Cinnamaldehyde	2*	18°					
Eugenol			1	9°*	10		
Piperine						2	18°*
Capsaicin			20°*				

° MIC for *B. hyodysenteriae* B78^T (ATCC 27164)
* MIC for *B. intermedia* PWS/A^T (ATCC 51140)

In an *in vivo* experiment, coated cinnamaldehyde was supplemented to the feed of rearing pullets. A completely randomized experimental design with 4 treatments and 3 replicates each (replicate = group of 7 one-day-old birds) was applied. The negative and positive control birds received a conventional feed during the whole trial. The positive control group was orally inoculated on 3 consecutive days (day 22, 23 and 24) with 1 ml 1.0 x 10^8 cfu/ml of a *B. intermedia* field isolate. Two treatment groups (preventive and curative), identically inoculated, were fed the coated cinnamaldehyde supplemented feed (final cinnamaldehyde concentration in the feed of 500 ppm), the preventive group from day 1 and the curative from day 25. On day 32, ceca were collected for bacteriologic *B. intermedia* enumeration.

RESULTS

The number of *Brachyspira*-positive birds and the mean enumeration of *Brachyspira* cells was decreased ($P < 0.05$) in the curative treated group versus the positive control group (Table 2).

Table 2

Bacteriologic enumeration of cecal *Brachyspira intermedia* in inoculated rearing pullets fed two diets differentially treated with coated *trans*-cinnamaldehyde

	Positive control group				Treatment[1]							
					Preventive group				Curative group			
Number of positive chickens (3 replicates)[2]	**10/21**	(3/7	4/7	3/7)	6/21	(2/7	3/7	1/7)	**1/21**	(0/7	0/7	1/7)
Mean enumeration in log10 cfu/g (3 replicates)[3]	**3.85**[a]	(3.79	4.96	2.79)	2.69[ab]	(2.64	4.30	1.14)	**1.29**[b]	(1.00	1.00	1.88)

[1]Treatment: Feed with 500 ppm *trans*-cinnamaldehyde; Preventive = fed all the study period long; Curative = fed only after *B. intermedia* inoculation.
[2]Positive chicken: when *Brachyspira* cells were demonstrated in the bacteriologic enumeration method with a detection limit of 100 cfu/g ceca.
[3]Mean of the results of bacteriologic quantification of *B. intermedia* in the ceca; birds under the detection limit of 2 log10 were given a log10 score of 1.
[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

CONCLUSIONS

- The *in vitro* results demonstrate the potential of EO components as antimicrobials against poultry *Brachyspira* isolates, including isolates with acquired resistance for classic antimicrobial drugs[1].
- Reduction of *Brachyspira* colonization in young pullets was obtained, on a curative way, in an *in vivo* study using feed supplemented with coated cinnamaldehyde.

References:
1. Verlinden, M., Boyen, F., Pasmans, F., Garmyn, A., Haesebrouck, F., and Martel, A. (2011). Antimicrobial susceptibility pattern of *Brachyspira intermedia* isolates from European layers. *Microbial Drug Resistance* 17: 485-488.
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